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Amino acid profile, Antioxidant Activity and flavonoid Content of White Lotus 'Purain' from North Bihar, India

B. R. Jana, Manoj Kumar and S.M. Raut

ICAR-National Research for Makhana, Darbhanga, Bihar, India
Email: brjana.ars@gmail.com

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***Abstract*— *India's national flower is the lotus. A lot of seed is carried by white lotuses. Protein and other bioactive substances are abundant in white lotus seeds. An experiment was carried out at the ICAR-National Research Centre in Makhana in 2023-24 to assess the nutritional quality and anti-oxidant characteristics of white lotus seed, which is abundant during the rainy and autumn seasons in the wetland ecosystem of north Bihar. Seeds of white purain accession were grinded and its flour chemically analyzed using AOAC standard methods. Estimation of amino acids was based on the reaction of free amino acids with phenyl isothiocyanate produces stable derivatives that are then separated using liquid chromatography. Anti-oxidant activities were estimated in spectrophotometric observation by DPPH and FRAP methods, based on electron transfer reactions, which visually results on the reduction of a coloured oxidant. Flavonoids are estimated by UHPLC methods. The white lotus purain seed content 17.8±054 % protein, 1.09±0.07 % fat and 1.94±0.08 % crude fibre. The nutritional study also revealed that the appreciable amount of arginine (12.70 g/kg) and histidine (3.62 g/kg) were present in the protein of the dry endosperm of Nelumbium spp. In addition, the lotus seed endosperm has a fair source of quercetin (422.0µg/kg) and kaempferol (200.0 µg/kg) as pure flavonoids which influence the rapid glucose metabolism in human cell. The greatest DPPH and FRAP values were found in seed endosperm of white lotus purain, which had 988.05±2.25 mg AEAC/100g FW and 461.78±1.79 mg AEAC/100g FW, respectively.*

***Keywords*— White lotus, amino acids, anti-oxidant activity, flavonoids**

I. INTRODUCTION

Lotus seeds are a fast snack to enjoy while when we are relax condition. It is sometimes referred to as lotus nut or kamal seed. The scientific name for lotus seeds is *Nelumbium spp* which comes from the lotus plant, *Nelumbium nucifera*. For almost 7000 years, it has been used as a useful food, medicine, and vegetable. There are two varieties of dried lotus seeds: white peel and brown peel. Lotus seeds are widely manufactured and cultivated in India, Japan, and China. Lotus seeds are low in calories and high in nutrients, making them an important part of the body's operations. Potential usage of lotus seed is for weight management. Adipocytes (fat cells) are responsible for the excess weight in the body. A 2011 study by **Achike et al.** found that lotus seeds may suppress fat cell development and reduce fat tissue weight. Additionally, polyphenols present in lotus seeds may improve the body's lipid profile.1 However, more research is needed to determine whether lotus seeds can assist manage weight. As a result, you must consult your dietitian before making any dietary adjustments. Lotus seeds may have neuroprotective properties, which means they may prevent nerve cells from injury in cases such as Alzheimer's disease. **Kim et al. (2014)** discovered that proanthocyanidins in lotus seeds may minimize brain aging and cognitive impairment. Lotus seeds may also lower the incidence of Alzheimer's disease by reducing damaging free radicals and the formation of unneeded calci-um.1 More research is needed to determine whether lotus seeds can help improve Alzheimer's disease symptoms. If you see any symptoms of Alzheimer's disease, you should see your doc-tor. **Sugimoto et al. (2008)** found that bioactive substances such as saponins, flavonoids, alkaloids, and tannins may aid to alleviate

anxiety and depression. Lotus seeds' alkaloids may help you sleep longer. Getting enough sleep may assist with depression. However, these experiments were carried out on mice.1 More research on humans is needed to determine whether lotus seeds are effective for anxiety and depression. According to **Tang et al.'s 2017** study, lotus seeds may have antimicrobial capabilities. Lotus seeds may alter the structure and function of the plasma membrane. Lotus seeds may also limit the growth of Salmonella, Staphylococcus aureus, Escherichia coli, Klebsiella sp., Shigella, and Pseudomonas sp.1. However, more research is needed to determine whether lotus seeds can be utilized to treat microbiological illnesses. If you develop a microbiological infection, you must visit your doctor. **Mani et al. (2010)** found that lotus seed could be utilized to treat type 1 and type 2 diabetes. Various nutrients found in lotus seeds may be useful to diabetics. Zinc in lotus seeds, for example, decreases oxidative stress in type 1 diabetes patients while transporting glucose to cells in type 2 diabetics. Chromium may boost insulin receptors and glucose metabolism, lowering blood glucose levels in type 2 diabetic patients.3 However, more research is needed to determine whether lotus seeds can help to reduce blood glucose levels. **Poornima et al. (2013)** discovered that lotus seeds may be effective against lung cancer. Nepherine, a bioactive chemical found in lotus seeds, may kill cancer-causing cells (apoptosis) and limit their prolifera-tion.4 However, more research is needed to determine whether lotus seeds can help prevent cancer. If you suspect cancer, you should visit your doctor rather than self-medicate. A 2009 study by **Chakravarthi et al.** found that the flavonoids in lotus seeds may aid to reduce dis-comfort. Lotus seeds may suppress the cyclooxygenase enzyme, which is responsible for the release of pain-related mediators.5 However, additional research is needed to determine whether lotus seeds can assist reduce discomfort. If you are experiencing continuous pain, you should visit your doctor. **Rai et al. (2006)** found that flavonoids in lotus seeds may be responsible for their antioxidant properties. Flavonoids may destabilize damaging free radicals (molecules in the body) and lessen oxidative stress by eliminating them. This would minimize the risk of various ailments, including diabetes, heart disease, and liver disease.1,6 However, additional re-search is needed to determine the antioxidant potential of lotus seeds.

The present study was conducted to evaluate the nutritional status of purain (white lotus) which is often grown in freshwater wetland in north Bihar, in order to promote its cultivation for dry seeds flowers.

II. MATERIALS AND METHODS

The experiment was conducted at RCM, Darbhanga, Bihar, during 2023-24. Dry lotus nut (*Nelumbium* spp.) kernels were smashed and ground into powder from four replications separately. White lotus purain genotype was taken for the research material as it is predominant in habitation under subtropical climate of north Bihar.

Proximate composition

Nutritive properties including moisture, ash, crude fat, and crude protein contents were deter-mined by AOAC methods **(A.O.A.C., 2016).** Crude protein was analyzed using Kelplus Elite Ex Micro Kjeldahl method **(Ranganna, 1997),** using conversion factor 6.25. Carbohydrates (by difference) % weight was determined as followed by the method of **Gopalan et al., (1998).** Total dietary fiber (TDF) was determined by digesting the sample with α-amylase (**AOAC method 991.43).** All the biochemical analyses were carried out in four replications using fresh and dry makhana kernel powder.

Amino acids

All essential amino acids were analyzed using the methods described by **Bidlingmeyer (1987).**

Anti-oxidant activity

The total free radical scavenging capability of extracts from various plant samples was calculated using the previously reported method **(Brand -Willium et al 1995)** with a little modification utilizing the stable DPPH radical, which has an absorption maximum at 515 nm. DPPH free radical scavenging activity was evaluated as ascorbic acid equivalent antioxidant capacity (AEAC). The antioxidant content of medicinal plants was evaluated spectrophotometrically using the procedure mentioned by **Benzie and Strain (1996).** The approach works by reducing the Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+} -tri-pyridyl-triazine (blue complex) at low pH using electron-donating antioxidants. This reaction is studied by observing the change in absorbance at 593 nm. FRAP free radical scavenging activity was evaluated as ascorbic acid equivalent antioxidant capacity (AEAC).

Flavonoid analysis

Ten grams of sample was homogenized with 70% methanol. Samples were vortexes for 5 min followed by centrifugation at 4,500 rpm for 10 mins. Filter the supernatant in 0.45 µm syringe filter. Then the samples were injected in HPLC system. Column oven temperature was 30.0 ^{0}C. Injected volume was 20 µL and flow rate was 1.0 ml/min. Mobile phase A was 6% acetic acid in 2 mM sodium acetate aqueous solution (v/v, final pH 2.55). On the other hand, the mobile phase B was Aceto-nitrile. The time gradient was, 0.01, 45.0, 60.0, 65.0, 70.0,75.0,

and 80.0 minutes (stop). Post run time was 5.0 minutes (**Tsao and Yang, 2003).** Before sample loading we run two standards separately with above mention column condition.

III. RESULTS AND DISCUSSION

Proximate composition

Data pertaining to table-1 revealed that makhana seed kernel contained 16.3±054 %, pro-tein, 1.94±0.08% crude fiber, 1.09±0.07 % total fat and 67.6±1.29 % carbohydrates. According to **Sathithon and Yan-bin (2012) and Shahzad et al. (2021)** the lotus seed has a moisture content of 5.0%–9.0%, 61%–62% carbohydrates, 16.0%–21.0% total protein, and 2.40%–3.0% crude fat. Starch makes up a significant portion of the carbohydrates found in lotus seeds. Simi-lar results in respect of proportion of proximate composition were also obtained by **Kumar et al., (2016)** and **Jana et al., (2021)** in other popular aquatic nut makhana. Lotus seeds are rich in nutrients, containing a well-balanced combination of minerals, lipids, carbohydrates, and proteins. The nutritional value of lotus seeds varies by species and growing region. Nearly comparable physiological characteristics and composition have been reported **(Shahzad et al., 2021).**

Table-1: Nutritional composition of makhana dry seeds endosperm of white Lotus , Purain

Sl. No.	Parameters	Proximate composition [(%) by weight]
1.	Moisture	10.45±0.62
2.	Total Ash	0.97±0.04
3.	Total fat	1.09±0.07
4.	Protein (N*6.25)	17.8±054
5.	Crude Fiber	1.94±0.08
6.	Carbohydrates (by difference)	64.6±1.29

****Results were expressed as the mean of triplicate measurements ± SD. Significance at (P<0.05)*

Amino acid profile

The results shown in the table-2, the endosperm of seed of the white purain seed produced the maximum amount of amino acids, aspartic acid (1.87±0.11%), glutamic Acid (1.82±0.07%), serine (1.29±0.06 %), histidine (0.39±0.04 %), glycine (1.08±0.03%), arginine (1.76±0.14%), alanine (1.21±0.12%). tyrosine (0.59±0.04%), cysteine (0.11±0.05%), valine (1.06±0.06%), methionine (0.37±0.03%), phenyl-alanine (1.97±0.13 %), isoleucine (0.86±0.09 %) and leucine (1.53±0.07%). Similar type of results regarding different amino acids presence i.e. composition in makhana was confirmed by **Jana and Idris (2018).** Lotus seed contains essential and non-essential amino acids. In 1973, The Joint FAO/WHO expert committee has reported that the essential amino acids make 36% of the total amino acids available in lotus seeds.

White Lotus Purain (Plate 1-2)

Table-2: Amino acid profiles of dry seeds endosperm of white lotus, Purain

Sl. No.	Amino acids	Quantity of amino acids (%) in 100g seed kernel
1.	Aspartic acid	1.87±0.11
2.	Glutamic acid	1.82±0.07
3.	Serine	1.29±0.06
4.	Glycine	1.08±0.03
5.	Histidine	0.39±0.04
6.	Arginine	1.76±0.14
7.	Alanine	1.21±0.12
8.	Proline	0.75±0.05
9.	Valine	1.06±0.06
10.	Methionine	0.37±0.03
11.	Cysteine	0.11±0.05
12.	Isoleucine	0.86±0.09
13.	Leucine	1.53±0.07
14.	Lysine	1.08±0.09
15.	Phenylalanine	1.97±0.13
16.	Tyrosine	0.59±0.04
17.	Tryptophan	n.d.
18.	Threonine	1.68±0.09
19.	Total	19.42±0.074

****Results were expressed as the mean of triplicate measurements ± SD. Significance at ($P<0.05$).*

Anti-oxidant activity

Antioxidants are mainly defined as polyphenolic compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals (ROS) and diminishing oxidative stress of the cell. The reducing capacity of a compound may serve as an indicator of its potential antioxidant capacity. The DPPH free radical, can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule. On the other hand, FRAP values; this might be due to reduced ferric ion and the high chlorogenic acid content. White lotus purain had the highest DPPH and FRAP values of 988.05±2.25(mg AEAC/100gFW, and 461.78±1.79 mg AEAC/100g FW, respectively (Table-3). Thus, makhana is a rich source of several antioxidants, which are helpful against several metabolic disorders.

Table-3: Different anti-oxidant activity in dry seeds endosperm of white Lotus , Purain

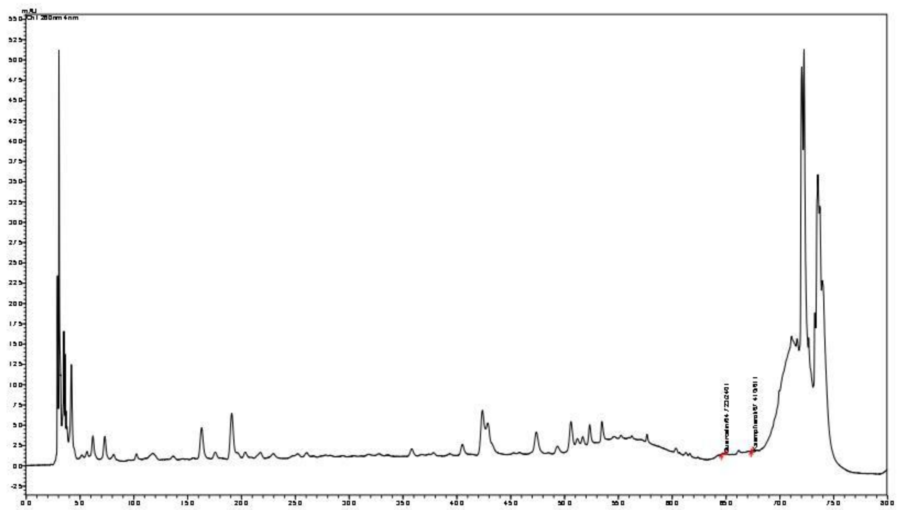
Sl.No.	Method of Determination of Antioxidant activity	Anti-oxidant activity
1.	DPPH	988.05±2.25(mg AEAC/100g)
2.	FRAP	461.78±1.79(mg AEAC/100g)

****Results were expressed as the mean of triplicate measurements ± SD. Significance at ($P<0.05$).*

Flavonoids

The flavonoid. quercetin, is a polyphenolic compound and the most abundant flavonoid in edible vegetables, fruit, wine and nuts. In the present experiment, flavonoids quercetin and kaempferol were estimated by using UHPLC chromatography in pure form. In the present experiment, the retention time for quercetin and kaempferol was 64.5 and 67.9 min., respectively. Furthermore, the peak area was 2401 units and 611 units, respectively. The presence of quercetin 0.523 mg/kg and kaempferol 0.087 mg/kg as pure form (Fig-1) without other derivatives of the two flavonoids was revealed by a UHPLC chromatogram of white purain seed endo-sperm. Other glycosidic compounds and esters may be present in the seed endosperm as many other peaks were also visible in Fig-2. Theses seeds may contain their other derivatives like quercetin and kaempferol glucosides and glucuronides or esters compounds. This findings were also observed corroborated by Zhu et al., (2016).

UHPLC chromatogram of Quercetin and Kaempferol (Code No: 804/Lotus seeds)



S. No	Name	RT (min)	Area	Results (mg/Kg)
1	Quercetin	64.5	2401	0.523
2	Kaempferol	67.9	611	0.087

Fig-1: Presence of quercetin and kaempferol flavonoids in white lotus seeds.

IV. CONCLUSIONS

The nutritional value, amino acid composition, flavonoids, and antioxidant activity of white lotus seed purain were assessed. The seed contained 19.85% protein and had a well-balanced amino acid composition when compared to the FAO/WHO pattern. The limiting amino acids in seed proteins were phenylalanine, tyrosine, leucine, and lysine. Overall, the study found that lotus seed protein was a nutritionally balanced protein that could play an important role in the composition of human diets. Lotus seed endosperm has several nutritional benefits, but its abundance in secondary metabolites like flavonoids and alkaloids has sparked interest in its potential as a functional food.

CONFLICT OF INTEREST

Author declares that they have no conflict of interest.

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